

CALCULATE RESULTS

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

$$\%B^{\circ} = (\text{average OD of calibrator, control or sample} \times 100) \div \text{average OD of negative control}$$

2. Graph the %Bo of each calibrator on the Y (linear) axis against its concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.
3. Determine the Capsaicin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph and multiply by the appropriate dilution factor.
4. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

SAMPLE CALCULATIONS

Well Contents	OD	Average OD \pm SD**	%RSD	%Bo	Capsaicin Conc. (ppm)
Negative Control	1.574 1.533	1.553 \pm 0.029	1.86	100	N/A
0.1 ppm Calibrator	1.250 1.281	1.265 \pm 0.022	1.73	81	N/A
0.5 ppm Calibrator	0.764 0.739	0.751 \pm 0.018	2.35	48	N/A
2.0 ppm Calibrator	0.354 0.335	0.344 \pm 0.013	3.91	22	N/A
Sample	0.858 0.877	0.868 \pm 0.013	1.55	56	0.35

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302

SAFETY

To receive complete safety information on this product, contact Beacon Analytical Systems, Inc. and request Material Safety Data Sheets. Stop Solution is 1N hydrochloric acid. Handle with care.

General Limited Warranty

Beacon Analytical Systems, Inc. ("Beacon") warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product's printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon's sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.



Capsaicin Plate Kit

Cat.# 20-0072

Instructional Booklet

READ COMPLETELY BEFORE USE.

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INTENDED USE

The Beacon Capsaicin Plate Kit is an immunological laboratory test for the quantitation of Capsaicin in raw peppers and salsa.

USE PRINCIPLES

The Beacon Capsaicin Plate Kit uses a polyclonal antibody that binds both Capsaicin and a Capsaicin-enzyme conjugate. Capsaicin in the sample competes with the Capsaicin-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind Capsaicin, are immobilized to the inside of the test wells. In the assay procedure you will:

- Add a mixture of a sample containing Capsaicin and Capsaicin-enzyme conjugate to a test well. The conjugate competes with any Capsaicin in the sample for the same antibody binding sites.
- Wash away any unbound molecules, after you incubate this mixture for 10 minutes.
- Add clear substrate solution to each well. In the presence of bound Capsaicin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of Capsaicin-enzyme conjugate molecules, a sample containing a low concentration of Capsaicin allows the antibody to bind many Capsaicin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of Capsaicin allows fewer Capsaicin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to Capsaicin concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

MATERIALS PROVIDED IN THE BEACON CAPSAICIN PLATE KIT

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

This plate kit contains the following items:

- 1 plate containing 8 strips of 12 wells coated with rabbit anti-Capsaicin antibodies
 - 1 bag containing 12 strips of mixing wells
 - 1 vial of Negative Control (0.0 ppm Capsaicin)
 - 1 vial each of 0.1 ppm, 0.5 ppm, and 2.0 ppm Capsaicin (natural mixture) Calibrator
 - 1 vial of Capsaicin-HRP Enzyme Conjugate
 - 1 vial of Substrate
 - 1 vial of Stop Solution
 - 1 Instructional Booklet
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PERFORMANCE CHARACTERISTICS

SPECIFICITY

The Beacon Capsaicin Plate Kit is specific for Capsaicin with reactivity to a limited number of closely related compounds. The following table shows the relative values for 50% B₀ and the percent cross-reactivity (%CR) versus Capsaicin (natural). All concentrations are in parts per million (ppm).

Compound	50% B ₀	%CR
Capsaicin (natural mixture)*	0.625	100
Capsaicin (pure)	0.599	104
Dihydrocapsaicin	0.639	98

*Contains ~ 65 % capsaicin and 35 % dihydrocapsaicin

MATERIALS REQUIRED BUT NOT PROVIDED

- Laboratory quality distilled or deionized water.
- Methanol, ACS grade
- Graduated cylinder, 100 ml or larger.
- Glassware for sample extraction and extract collection.
- Pipet with disposable tips capable of dispensing 100 μL.
- Multi-channel pipet; 8 channel capable of dispensing 50 and 100 μL.
- Paper towels or equivalent absorbent material.
- Microwell plate or strip reader with 450nm filter.
- Timer

PRECAUTIONS

- Each reagent is optimized for use in the Beacon Capsaicin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Capsaicin Plate Kits with different Lot numbers.
 - Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
 - Do not use reagents after expiration date. Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).
 - Reagents should be brought to room temperature, 20 – 28°C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
 - The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
 - Use approved methodologies to confirm any positive results.
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SAMPLE PREPARATION

1. Puree a representative sample of salsa or raw pepper in a blender for 2 minutes to ensure a homogeneous sample.
2. Weigh 5 grams of the pureed sample into a 50 ml conical centrifuge tube and add 25 ml methanol.
3. Homogenize the mixture using a Polytron for 3 minutes at medium speed.
4. Centrifuge for 10 minutes at 15,000 x g. Remove and save supernatant.
5. Dilute supernatant 1:10 in laboratory grade water. If further dilutions are required to bring the sample concentration within the range of the curve, serially dilute in 10 % methanol/water.

Note: For dried pepper and oleoresin samples, we recommend Beacon's Capsaicin-HS assay.

ASSAYPROCEDURE

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow all kit reagents and samples to warm to room temperature.
 2. Remove the required number of red labeled mixing wells from plastic bag. Remove an equal number of antibody coated strips from the re-sealable foil bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
 3. **Pipet 100 μL of calibrators or samples** into the appropriate mixing wells. Be sure to use a clean pipet tip for each solution to avoid cross contamination.
 4. Add **100 μL of Enzyme Conjugate** to each mixing well.
 5. Mix the contents of each well gently by pipetting up and down a few times with a multichannel pipetter, then transfer **100 ul** of the mixture to the antibody coated reactions wells.
 6. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotater for continuous mixing during incubation.
 7. Incubate for **10 minutes**. Discard mixing wells.
 8. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
 9. Add **100 μL of Substrate to each well**.
 10. Cover the wells and incubate for **10 minutes**.
 11. Add **100 μL of Stop Solution** to each well in the same order of addition as the Substrate.
 12. Read the plate on a microtiter plate reader at 450nm. If the plate reader has dual wavelength capability, read at 450nm minus 605 or 650nm.
 13. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4-parameter curve fit. If manual data reduction is required, proceed as in the calculate results section.
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